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Infectivity of Respiratory Syncytial Virus by Various Routes of Inoculation

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To understand the transmission of respiratory syncytial virus, we examined the frequency of infection in volunteers after inoculation by different routes with varying doses of virus. Thirty-two adult volunteers received serial dilutions of a safety-tested live strain of respiratory syncytial virus instilled into nose, eye, or mouth. The highest inoculum, 5.2 $\log_{10}$ 50% tissue culture infective dose ($TCID_{50}$), was administered to four groups of four subjects each, by nose to one group, by eye to one group, and by mouth to two groups. Subsequently, 1:100 and 1:1,000 dilutions of this inoculum were administered by nose and eye. At the highest inoculum, infection occurred in three of four subjects inoculated by nose and in three of four subjects inoculated by eye. Infection occurred in one of eight subjects inoculated by mouth, but this subject most likely was infected by secondary spread. With an inoculum of 3.2 $\log_{10}$ $TCID_{50}$, the proportion of subjects infected by either route diminished to 25%. When the inoculum was further reduced to 2.2 $\log_{10}$ $TCID_{50}$, no infections occurred by either route. Infections after the highest inoculum were characterized by earlier and greater shedding. These findings suggest that respiratory syncytial virus may infect by eye or nose and that both of these routes appear equally sensitive. In comparison, the mouth appears to be an insensitive route of inoculation. This is of potential import in infection control procedures and in the development of vaccines or other prophylactic measures.

Respiratory syncytial virus (RSV) appears to be highly contagious and spreads effectively among groups of young children, in families and on hospital wards (5, 6, 10). On pediatric wards it has become the major nosocomial hazard, resulting in appreciable morbidity and mortality (3-5, 7, 8, 15). Infection control procedures have been of only limited benefit in curtailing the spread of RSV. In part, this may result from our lack of understanding of its mechanisms of transmission. Determination of the possible routes of inoculation and their relative sensitivity could aid in the development of prophylactic measures, whether mechanical, chemotherapeutic, or immunological. This study, therefore, was designed to determine the relative infectivity of RSV administered by nose, eye, and mouth in adult volunteers.

MATERIALS AND METHODS

Volunteers. The volunteers for this study were young adults who were determined to be in good health and free of atopic manifestations by physical examination, history, chest roentgenogram, and laboratory tests that included a complete blood cell count, SMA-12, and a pregnancy test for women volunteers. In addition, base-line pulmonary function studies were performed. From each volunteer, informed consent was obtained as approved by the human investigation committees of both the University of Rochester and the National Institute of Allergy and Infectious Diseases.

The volunteers were isolated for 10 days from the time of inoculation of the virus. During this time, physical examinations were performed twice daily, histories were obtained, and findings were recorded by a physician who was not aware of the route of administration of the virus. Temperatures were taken and recorded four times each day. Nasal washes were obtained before inoculation, subsequently twice each day during the period of isolation, and again 4 weeks after inoculation. Serum was obtained from each volunteer before inoculation and subsequently at 10 days and 4 weeks. Pulmonary function testing was obtained on any volunteer developing respiratory symptoms.

Virus stock and administration. The virus administered was a safety-tested, live RSV stock obtained through the courtesy of Robert Chanock at the National Institutes of Health. This was derived from RSV strain A-2, which was multiply passaged in the following tissue cultures: human embryonic kidney (6 passages), calf kidney (10 passages), and bovine embryonic kidney (4 passages). Undiluted stock (1 ml) was administered initially to one group of volunteers by nose drops, 0.5 ml per nostril, with the subject in the supine position. To a second group, 1.0 ml of the

undiluted stock was inoculated into the eye by administering one drop (0.05 ml) every 5 to 10 min into both conjunctival sacs. A third group were given virus by mouth by dropping 1.0 ml onto the tongue. Subsequently, 1:100 and 1:1,000 dilutions of this virus stock were administered to groups of volunteers into the eye and nose. Each time the virus stock was administered to a volunteer, titers were concurrently determined, and it was shown to contain an average of 5.2 $\log_{10}$ tissue culture infective dose ($TCID_{50}$) per ml (range, 5.1 to 5.7 $\log_{10}$ $TCID_{50}$ per ml).

Viral isolation. A portion of each nasal wash was immediately inoculated onto HEp-2 cell cultures for viral isolation, and the titer was estimated by inoculation of serial 10-fold dilutions onto HEp-2 cell cultures. Additional cell lines (Rhesus monkey kidney, WI-38, and Madin-Darby canine kidney [MDCK]) were utilized in an attempt to isolate other viruses from the nasal washes obtained before inoculation and from washes from volunteers who developed signs of illness.

Serum and nasal antibody determinations. The titers of RSV antibodies in paired sera of volunteers were determined by complement fixation test (1), neutralization test with complement added (2), and enzyme-linked immunosorbent assay (17, 18). Immunoglobulin A (IgA) antibody against RSV in the nasal secretions was determined before inoculation and subsequently at 10 days and 4 weeks by the indirect immunofluorescent technique of McIntosh et al. (13). Total secretory IgA was measured by single radial immunodiffusion, and IgA antibody titers against RSV were calculated per 10 mg of total IgA per 100 ml.

Pulmonary function testing. Forced expiratory flow rates were measured by clinical spirometry and flow-volume curve determinations.

RESULTS

Thirty-two subjects were included in the study. As shown in Table 1, 12 subjects were inoculated by nose, 12 were inoculated by eye, and 8 were inoculated by mouth. In the groups receiving the highest inoculum by nose and by eye, three of four became infected, as demonstrated by shedding of RSV. When the dose was reduced 100-fold, one of four subjects with eye inoculation and one of four subjects with nasal inoculation became infected. Further reduction of the inoculum by 10-fold produced no detectable infection in either of these two groups. Of the initial four subjects inoculated by mouth, one shed RSV. However, this subject may have become secondarily infected since the incubation period was twice as long as for the others receiving this dose. Furthermore, this volunteer was found to have not followed the isolation procedures and socialized with one of the other volunteers, who was shedding virus on day 3.

The patterns of shedding were similar for subjects receiving the same dose whether they were inoculated by nose or eye (Fig. 1 and 2). Subjects infected by the highest inoculum began shedding the virus on day 3 to 4. Reducing the inoculum by two logs delayed the onset of shedding until day 7, and the duration and quantity of shedding was diminished. The one subject in the group inoculated by mouth, who may have become infected secondarily, shed virus on days 6 to 10, with titers up to 2.5 $\log_{10}$ $TCID_{50}$ per ml.

TABLE 1. *Comparative rate of infection in volunteers inoculated with RSV by various routes and doses*

Inoculation route	Dose (Log_{10} $TCID_{50}$)	No. of subjects:				
		Inoculated	Shedding RSV	With seroresponse		
				CF[a]	NT[b]	ELISA[c]
Nose	5.2	4	3	2	3	3
	3.2	4	1	1	1	1
	2.2	4	0	0	0	0
Eyes	5.2	4	3	0	2	3
	3.2	4	1	0	0	0
	2.2	4	0	0	0	0
Mouth	5.2	8	1[d]	1	1	1

[a] CF, Complement fixation test, with seroresponse defined as ≥ fourfold rise in titer.

[b] NT, Neutralization test, with seroresponse defined as ≥ fourfold rise in titer.

[c] ELISA, Enzyme-linked immunosorbent assay. Response defined as E ratio (absorbance of post-inoculation serum at 1:100 over absorbance of pre-inoculation serum) of ≥ 1.22 (18).

[d] Infected probably by secondary spread.

A serological response was detected by one or more of the three antibody assays in eight of nine subjects who shed RSV (Table 1). No subject had a serological response who did not also demonstrate viral shedding. The pre-inoculation antibody titer did not appear to relate to the chance of a subject becoming infected (Table 2).

All of the nine infected subjects had a two- to fourfold rise in specific nasal IgA antibody. In five, the rise was fourfold or greater, and in four, it was twofold. Of the 23 subjects who did not shed virus, 19 had no detectable rise in IgA antibody; one who was inoculated with the highest dose by nose had a fourfold rise, and three had a twofold rise who were inoculated by eye, one with the highest dose and two with the intermediate dose. None of the subjects, however, was symptomatic. The presence of specific IgA antibody in the nasal secretions before inoculation of the virus could not be related to the chance of subsequent infection. Three of the nine subjects who became infected had specific nasal antibody present in these secretions before inoculation, as did 3 of the 23 uninfected subjects.

Eight of the nine infected subjects developed

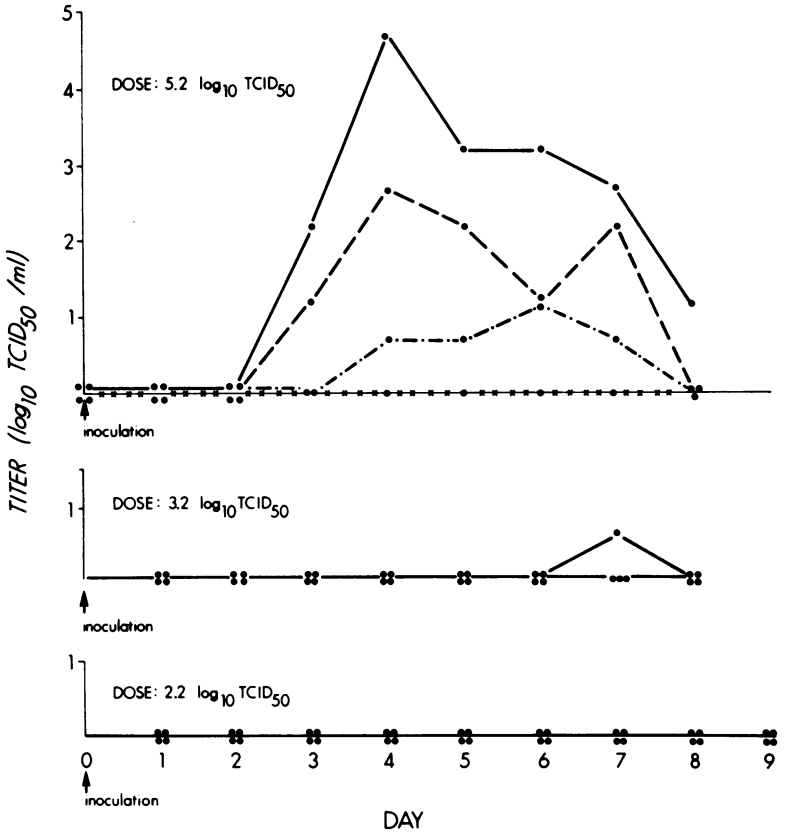

FIG. 1. *Daily frequency and quantity of viral shedding in 12 volunteers inoculated intranasally with RSV are shown, relative to inoculating dose.*

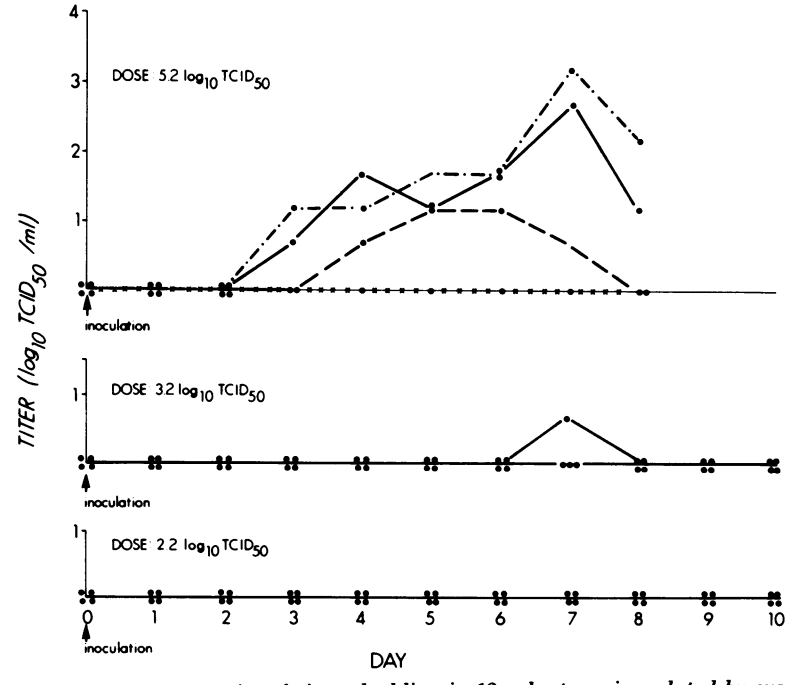

FIG. 2. *Daily frequency and quantity of virus shedding in 12 volunteers inoculated by eye with RSV are shown, relative to inoculating dose.*

TABLE 2. *Mean and range of the antibody titers before and after inoculation of RSV in infected subjects compared with uninfected subjects*

Serological test	Serological titers of subjects			
	Infected		Uninfected	
	Pre-inoculation	Post-inoculation	Pre-inoculation	Post-inoculation
Complement fixation				
Geometric mean	3.7	12.7	5.6	5.4
Range	<4-32	4-32	<4-16	<4-16
Neutralization				
Geometric mean	20.1	59.3	17.5	18.0
Range	8-64	32-128	8-64	8-64
ELISA				
Mean[a]	8.17	10.87	9.24	8.58
Range[a]	3.7-14.4	6.9-15.4	5.6-18.8	5.8-14.8

[a] Absorbance (optical density) of serum at 1:100 dilution (18). ELISA, Enzyme-linked immunosorbent assay.

mild upper respiratory tract symptoms consisting of nasal stuffiness, sneezing, and sore throat or malaise, which lasted for 1 to 3 days. In two subjects, an oral temperature of 37.8°C was documented on 1 day. Cough, conjunctivitis, and pharyngitis were not observed. Of the two volunteers infected with the lower inoculum, one was asymptomatic and the other had slight rhinitis for 1 day. One other subject, who received the highest inoculum by nose, developed a mild rhinitis and sore throat on day 6. However, no agent was identified. Pulmonary function testing on all of the infected subjects showed no change from their base-line studies.

DISCUSSION

These findings indicate that transmission of RSV may occur as easily by the inoculation of the eye as by nose. The mouth, in contrast, appears to be a relatively insensitive route for infection. The characteristics of the resulting infection appeared similar whether inoculation was by eye or nose, if the dose was equivalent. The size of the inoculum for these two routes appeared to be the important factor in determining the onset, degree, and duration of shedding. The presence of specific nasal IgA antibody before inoculation could not be shown to be protective at these doses, nor did the level of serum antibody at the time of inoculation correlate with protection, a phenomenon that has been well described for RSV (11, 12).

The 50% human infectious dose for this RSV strain appeared to be about 4 $\log_{10}$ $TCID_{50}$ per ml when given by nose or mouth. However, Mills and co-workers (14), using this same RSV strain, were able to infect 100% of volunteers given $10^{2.7}$ plaque-forming units by intranasal inoculation and by nebulization into the nasal pharynx. However, when 10^5 plaque-forming units were similarly given, only half of the volunteers became infected. The reasons for the discrepancy in their study are not clear. Differences between their study and ours may relate to method of inoculation and age of the virus stock.

The human infectious dose for a wild strain of RSV most likely would be less than for this multiply passaged RSV strain. In support of this is an earlier study of second passage of a wild RSV strain which infected 83% of seropositive adult volunteers when administered in doses of 160 to 640 $TCID_{50}$ (12). Trials of attenuated RSV vaccines administered to seropositive children have shown that most were infected with 10^4 $TCID_{50}$ intranasally. However, one of three seronegative infants was infected by a much lower dose (30 to 40 $TCID_{50}$) of the ts-1 mutant RSV vaccine (16). It is likely that the dose of wild RSV required to infect an infant would be even less. In natural infections, initiation of infection probably commonly occurs with a small dose. This is suggested in this study by the lower inoculating doses resulting in a longer incubation period, which more closely mimics that observed in family studies of the spread of RSV (6).

The RSV strain used in our study also appeared attenuated clinically. Even at the highest inoculum, the resulting illness was mild, and no changes could be detected upon pulmonary function testing, in contrast to adults with natural RSV illness (9).

The practical importance of the findings of this study is that in any prophylactic measures developed against RSV, whether chemotherapeutic or mechanical, both portals of entry, the nose and eye, must be considered. RSV appears to be spread by large droplets, requiring close contact, and by self-inoculation after touching contaminated skin or objects, but not by small particle aerosols (C. B. Hall and R. G. Douglas, J. Pediatr., in press) . Infection control procedures have been of only limited value in controlling the nosocomial spread of this virus and particularly in preventing infection in hospital personnel (7). In part, this may be because there is no method of infection control that protects both the eyes and the nose from viral inoculation. Masks cover only one of the two potentially sensitive routes for RSV inoculation. Protection of the eyes and the nose, as by goggles, might be a more effective means of interrupting the transmission of RSV from infected infants to hospital personnel.

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LITERATURE CITED

1. **Beem, M., and D. Hamre.** 1969. Respiratory syncytial virus, p. 419–503. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Association, New York.
2. **Buynak, E. B., R. E. Weibel, A. J. Carlson, A. A. McLean, and M. R. Hilleman.** 1979. Further investigation of live respiratory syncytial virus vaccine administered parenterally. Proc. Soc. Exp. Biol. Med. **160:** 272–277.
3. **Ditchburn, R. K., J. McQuillin, P. S. Gardner, and S. D. M. Court.** 1971. Respiratory syncytial virus in hospital cross-infection. Br. Med. J. **3:**671–673.
4. **Goldson, E. J., J. T. McCarthy, M. A. Welling, and J. K. Todd.** 1979. A respiratory syncytial virus outbreak in a transitional care nursery. Am. J. Dis. Child. **133:** 1280–1282.
5. **Hall, C. B., R. G. Douglas, Jr., J. M. Geiman, and M. K. Messner.** 1975. Nosocomial respiratory syncytial virus infections. N. Engl. J. Med. **293:**1343–1346.
6. **Hall, C. B., J. M. Geiman, R. Biggar, D. I. Kotok, P. M. Hogan, and R. G. Douglas, Jr.** 1976. Respiratory syncytial virus infections within families. N. Engl. J. Med. **294:**414–419.
7. **Hall, C. B., J. M. Geiman, R. G. Douglas, Jr., and M. P. Meagher.** 1978. Control of nosocomial respiratory viral infections. Pediatrics **62:**728–732.
8. **Hall, C. B., A. E. Kopelman, R. G. Douglas, Jr., J. M. Geiman, and M. P. Meagher.** 1979. Neonatal respiratory syncytial virus infection. N. Engl. J. Med. **300:** 393–396.
9. **Hall, W. J., C. B. Hall, and D. M. Speers.** 1978. Respiratory syncytial virus infections in adults: clinical, virologic and serial pulmonary function studies. Ann. Intern. Med. **88:**203–205.
10. **Henderson, F. W., A. M. Collier, W. A. Clyde, Jr., and F. W. Denny.** 1979. Respiratory syncytial virus infections, reinfections and immunity: a prospective longitudinal study in young children. N. Engl. J. Med. **300:** 530–534.
11. **Johnson, K. M., R. M. Chanock, D. Rifkind, H. M. Kravetz, and V. Knight.** 1961. Respiratory syncytial virus. V. Correlation of virus shedding, serologic response and illness in adult volunteers. J. Am. Med. Assoc. **176:**663–637.
12. **Kravetz, H. M., V. Knight, R. M. Chanock, J. A. Morris, K. M. Johnson, D. Rifkind, and J. P. Utz.** 1961. Respiratory syncytial virus. III. Production of illness and clinical observations in adult volunteers. J. Am. Med. Assoc. **176:**657–663.
13. **McIntosh, K., H. B. Masters, I. Orr, R. K. Chao, and R. M. Barkin.** 1978. The immunologic responses to infection with respiratory syncytial virus in infants. J. Infect. Dis. **138:**24–32.
14. **Mills, J., J. E. VanKirk, P. F. Wright, and R. M. Chanock.** 1971. Experimental respiratory syncytial virus infection of adults. J. Immunol. **107:**123–130.
15. **Mintz, L., R. A. Ballard, S. H. Sniderman, R. S. Roth, and W. L. Drew.** 1979. Nosocomial respiratory syncytial virus infections in an intensive care nursery: rapid diagnosis by direct immunofluorescence. Pediatrics **64:** 149–153.
16. **Parrott, R. H., H. W. Kim, C. D. Brandt, and R. M. Chanock.** 1975. Potential of attenuated respiratory syncytial virus vaccine for infants and children. Dev. Biol. Stand. **28:**389–399.
17. **Richardson, L. S., R. H. Yolken, R. B. Belshe, E. Camargo, H. W. Kim, and R. M. Chanock.** 1978. Enzyme-linked immunosorbent assay for measurement of serological response to respiratory syncytial virus infection. Infect. Immun. **20:**660–664.
18. **Steinhoff, M. C., C. B. Hall, and K. C. Schnabel.** 1980. Respiratory syncytial virus serology by a simplified enzyme-linked immunosorbent assay. J. Clin. Microbiol. **12:**447–450.